RESEARCH HIGHLIGHT

EMP1$^+$ tumor cells drive metastatic relapse

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Disease relapse after surgical management in patients with early-stage colorectal cancer remains a significant clinical challenge due to its high incidence and lack of efficacious therapeutic interventions. In a recent study, Canellas-Socias et al. demonstrate that EMP1$^+$ tumor cells are responsible for metastatic relapse and can be targeted by neoadjuvant immunotherapy to improve outcomes.

Minimal residual disease (MRD) describes a clinical state in which patients have no clinical or radiographic evidence of disease after surgical or medical treatment but continue to harbor undetected neoplastic cells. These residual tumor cells ultimately result in disease relapse, as observed in patients with early-stage colorectal cancer (CRC) who relapse after curative surgical interventions.[1] Serial monitoring of circulating tumor DNA (ctDNA) is a valuable tool to detect patients who will ultimately relapse due to MRD, and is currently being used in clinical trials to identify patients with MRD who receive additional therapeutic interventions to improve outcomes.[2] However, MRD-focused therapies have largely been exploratory due to the lack of available scientific knowledge to guide optimal targeting of residual disease. Despite the newly acquired ability to diagnose MRD in patients, further advancements to prevent metastatic relapse have been hindered by limited understanding of residual tumor cell biology and their vulnerabilities to therapeutic targeting.

LGR5, a Wnt pathway signaling receptor, is known to denote a subset of cancer stem cells (CSCs) in CRC which were suspected to be involved in tumor initiation, metastasis and relapse.[3] Studies investigating the role of LGR5$^+$ CSCs revealed that they are required for metastasis formation and growth, but surprisingly tumor cells that disseminate and seed metastases were shown to have an LGR5$^-$ phenotype.[4,5] With the source of metastatic relapse still unclear, Canellas-Socias et al.[6] sought to identify the key cells initiating metastasis in CRC.

To that end, the authors generated an epithelial-specific high-risk of relapse (EpiHR) gene signature from patients with CRC and used it to denote a subset of tumor cells, termed High-Risk Cells (HRCs), that express the poor prognosis transcriptomic program. A model of metastatic relapse was established using murine CRC (AKTP) tumor-derived organoids[7] to form cecal primary tumors, which were resected, and the mice were monitored as they developed metastases (Fig. 1a). Single-cell analysis of resulting primary tumors revealed a population of EpiHR^{High} cells, which were transcriptomically distinct from LGR5$^+$ CSCs, confirming the presence of HRCs in the murine model. With the ability to recapitulate micrometastasis and macrometastasis, the evolution of metastasis was investigated at the single-cell level showing that in micrometastases HRCs give rise to LGR5$^+$ CSCs, suggesting that HRCs drive metastatic relapse.

Additional analysis found that HRCs highly expressed EMP1, which could therefore be used to denote HRCs and validate their functional role. As such, to visualize and selectively deplete HRCs, a fluorescent reporter and inducible caspase-9 (iCas9)[8] cassette was knocked in to the *EMP1* locus of AKTP organoids, and used in their metastatic relapse model (Fig. 1a). The results showed that EMP1$^+$ HRCs were enriched in micrometastases but decreased as metastases grew, which overlaps with an increase in LGR5$^+$ CSC prevalence. Furthermore, ablation of EMP1^{iCasp9} cells successfully depleted primary tumor EMP1$^+$ HRCs, and when performed before tumor resection, it significantly decreased the metastatic relapse rate to 23% (5/22) compared to 79% (26/33) in control mice. Interestingly, EMP1$^+$ HRC ablation had no impact when performed after tumor resection or metastatic seeding had occurred.

With the specific role identified of EMP1$^+$ HRCs in metastasis initiation, the authors turned their attention to tumor microenvironment (TME) changes throughout the metastatic process. Primary tumors lacked tumor-infiltrating lymphocytes with the majority of CD3$^+$ T-cells found at the periphery. However, micrometastases revealed high T-cell density, which decreased as metastases grew and the TME complexity increased, indicating a naïve TME at metastasis initiation. HRCs in micrometastases had elevated PD-L1 expression, explaining the lack of immune activation and suggesting that immune checkpoint inhibitors (ICIs) may be uniquely effective in the underdeveloped TME of micrometastases. As such, anti-PD-L1 and anti-CTLA4 therapy prior to tumor resection decreased metastatic relapse rate to 22% (2/9) compared to 76% (13/17) in control mice. Similar to EMP1$^+$ HRC ablation studies, ICIs had no impact when administered after resection, confirming that neoadjuvant immunotherapy decreases metastatic HRC dissemination and seeding, but is unable to address established metastases.

The work of Canellas-Socias et al. provides pivotal insights into the mechanisms of MRD by identifying residual EMP1$^+$ tumor cells as metastasis-initiating cells and establishing a timeframe in which ICI therapy is effective for early-stage CRC (Fig. 1b). Together with previous LGR5$^+$-CSC findings, these results expand our understanding of metastasis and pave the way for the development of interventions targeting the disease course. Future studies addressing questions raised by this work would help to further gauge its impact on the field. These include exploration of additional HRC populations, not described by EMP1, that may contribute to the development of metastasis relapse; determining whether EMP1$^+$ HRCs are present across CRC subtypes, such as KRAS- and BRAF-mutated tumors, which would impact the scope of applicability of these findings; and, investigating whether EMP1$^+$ HRCs are specific to CRC or involved in the relapse of other cancer types (e.g., gastric, pancreatic, etc.).

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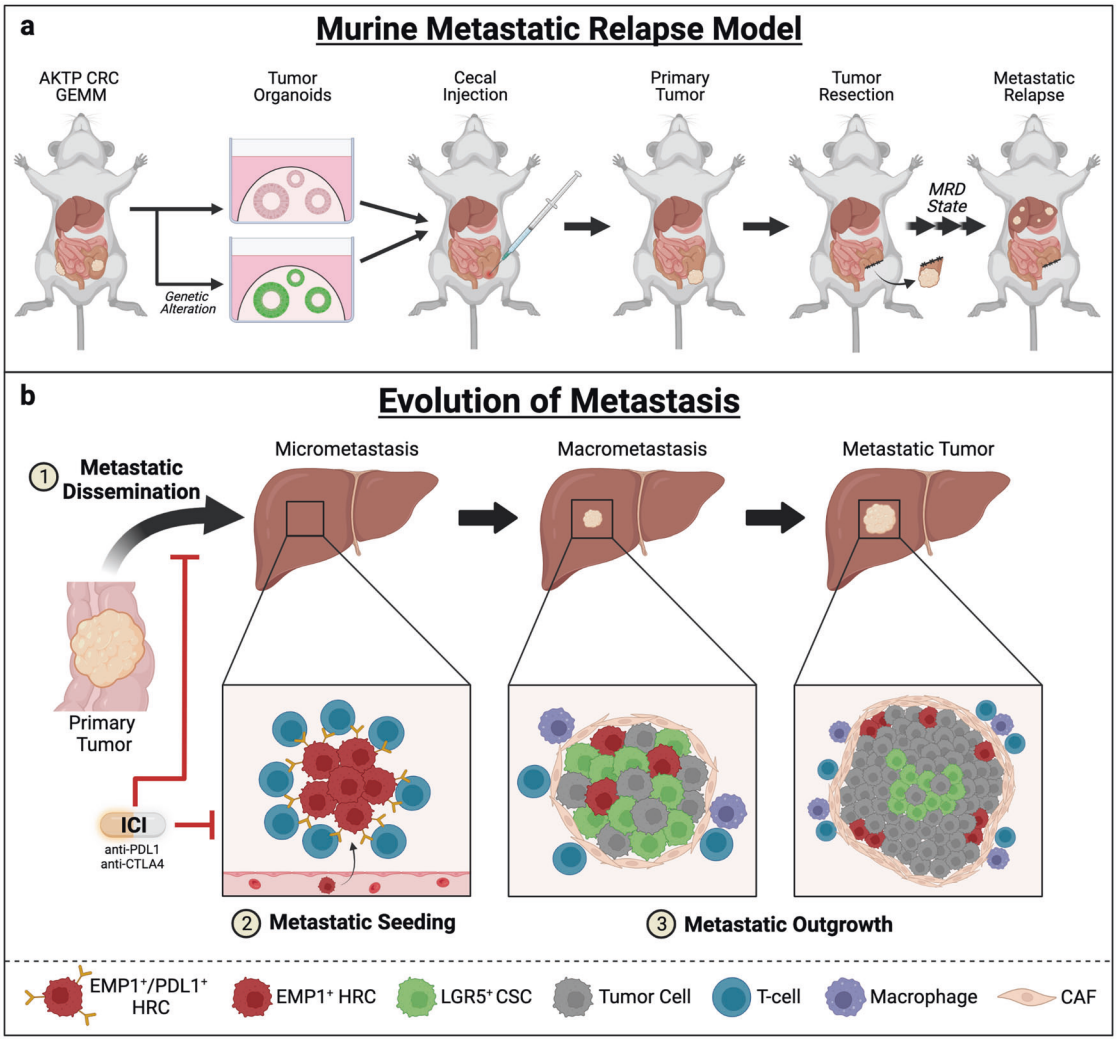

Fig. 1 Metastatic relapse in CRC. a Organoids from AKTP ($Apc^{fl/fl}$, $Kras^{LSL\text{-}G12D}$, $Tgfbr2^{fl/fl}$, $Trp53^{fl/fl}$) genetically engineered mouse model (GEMM)[6] tumors were injected orthotopically to establish cecal primary tumors; after tumor resection, mice developed liver metastases. EMP1/LGR5 reporter models were produced by genetic manipulation of organoids using CRISPR/Cas9. **b** Summary of metastasis development model. Disseminated tumor $EMP1^+$ HRCs seed micrometastases, which are composed of PD-$L1^+$ HRCs and have high T-cell density. During metastatic outgrowth, $EMP1^+$ HRCs produce $LGR5^+$ CSCs and the TME matures, recruiting cancer-associated fibroblasts (CAFs) and macrophages, to become T-cell exclusive. Neoadjuvant ICI inhibits early metastasis development, preventing relapse.

Immunotherapy is approved for treatment of microsatellite instability high (MSI-H) or deficient mismatch repair (dMMR) metastatic CRC, which represents 5%–10% of cases.[9] However, the majority of patients have microsatellite stable (MSS) tumors, which are not responsive to immunotherapy.[10] Given that the present study utilized an MSS CRC murine model, these findings highlight a specific opportunity in which ICIs may be effective for MSS tumors. Although few studies have explored neoadjuvant immunotherapy in CRC, the NICHE study demonstrated a 30% pathologic response in those with MSS, despite the lack of ICI efficacy in studies for established metastatic disease, supporting further testing of ICI therapy for MSS tumors in the neoadjuvant setting.[11] Further research into the unique vulnerabilities of $EMP1^+$ cells is warranted, which would enable additional MRD-focused clinical trials to evaluate hypothesis-driven approaches to target MRD in CRC. Overall, the work of Canellas-Socias et al. contributes meaningful insights into metastasis-initiating cells and the metastatic process, which will allow for the field to begin unraveling MRD mechanisms, and will better inform steps towards the optimization of therapeutic regimens for preventing CRC metastatic relapse.

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ADDITIONAL INFORMATION

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